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Exogenous mannitol treatment stimulates bud development and extends vase life of cut snapdragon flowers

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ABSTRACT

With a view to understanding the role of soluble carbohydrates in flower development and vase life, cut snapdragon (*Antirrhinum majus* L.) cv. Yellow Butterfly spikes were treated with glucose, sucrose, sorbitol and mannitol, which is a major carbohydrate in them. Treatment with 10–500 mM mannitol markedly promoted flower bud development and stem growth of cut snapdragon. Stem growth accompanied with bud development was not observed in 250 mM glucose, sucrose and sorbitol treatments. Mannitol treatment extended the overall vase life of cut snapdragons more than the other carbohydrate due to the promotion of flower opening at upper spike part. A pulse-chase experiment with ^{14}C-glucose, ^{14}C-sucrose or ^{14}C-mannitol showed that mannitol was metabolized slower than glucose and sucrose, suggesting that the different effects of carbohydrates on flower opening and stem growth may be due to different ability to be metabolized. The dry weight of flowers was greater in sucrose- or glucose-treated spikes than in mannitol-treated spikes, but the dry weight of upper stem parts including spike tips was gradually increased by mannitol treatment, suggesting that marked stem growth is due to applied mannitol. This explanation is supported by a tracer experiment with ^{14}C-carbohydrates showing that accumulation of ^{14}C in spike tips was greater in the mannitol treatment than in the glucose treatment. Flow cytometry revealed that degradation of nuclei, a parameter of programmed cell death (PCD), was promoted by sucrose, glucose, or sorbitol treatment, but was suppressed by the mannitol treatment. Carbohydrate concentrations in spike tips were markedly increased by glucose, sucrose, and sorbitol treatments, but were only slightly increased by the mannitol treatment. Water potential and osmotic potential in the spike tips decreased rapidly with the sucrose or sorbitol treatments, but were only modestly decreased by the mannitol treatment. The results suggest that mannitol suppressed a decrease in osmotic potential of spike tips, resulting in the continuation of bud development.

1. Introduction

Cut flowers are usually placed under conditions below the light compensation point for photosynthesis, and thus, they do not assimilate much carbon by photosynthesis, and thereby lack reserve carbohydrate. Treating with the ubiquitous metabolic sugars glucose, fructose, or sucrose, and supplementing with antimicrobial compounds, extends the vase life of many cut flowers, including carnation (Paulin and Jamain, 1982), rose (Kuiper et al., 1995; Ichimura et al., 2006), and sweet pea (Ichimura and Hiraya, 1999). These metabolic sugars have generally been considered to be similarly effective in extending the vase life of cut flowers (Halevy and Mayak, 1981). However, Ketsa and Boonrote (1990) reported that glucose extended the vase life of cut *Dendrobium* flowers more than sucrose. Similarly, continuous treatment with glucose or fructose was found to be more effective in extending the vase life of cut rose flowers than continuous treatment with sucrose (Ichimura et al., 2006). These findings indicate that exogenous sugars affect the vase life of cut flowers differently.

Other than these ubiquitous metabolic sugars, the effects of some soluble carbohydrates occurring in plants on their flower opening and vase life have been studied. In rose, *myo*-inositol, which is present generally in higher plants (Anderson and Wolter, 1966; Loewus and Dickinson, 1982), is a major carbohydrate in leaves, whereas xylose and methyl glucoside are minor sugar constituents (Ichimura et al., 1997). Treatments with methyl glucoside and xylose promoted flower opening, but treatment with *myo*-inositol inhibited opening of cut rose flowers (Ichimura et al., 1999a,b). Mannitol is the major carbohydrate in *Delphinium*

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(Ichimura et al., 2000). Exogenous mannitol reduces sensitivity to ethylene, delays climacteric-like increases in ethylene production, and suppresses sepal abscission in cut *Delphinium*. These actions are observed with glucose, but are not observed with 3-*o*-methyl-glucoside, a non-metabolizable sugar (Ichimura et al., 2000).

Snapdragon is an important cut flower because of its wide range of petal colors and good fragrance. Snapdragons have an indeterminate inflorescence with flower buds that open from the stem base to the apex. However, the vase life of cut snapdragons is relatively short and limited by flower abscission, wilting, or failure of buds to fully open (Larsen and Scholes, 1966; Wang et al., 1977; Nowak, 1981). Most snapdragon cultivars are sensitive to ethylene-induced flower abscission and wilting (Ichimura et al., 2008), but pulse treatment with silver thiosulfate complex (STS), an inhibitor of ethylene action, does not markedly extend the vase life of cut snapdragons (Ichimura et al., 2008; Nowak, 1981). In contrast, continuous treatments with sucrose and glucose markedly promote flower opening, thereby extending the overall vase life of cut snapdragon spikes (Ichimura and Hisamatsu, 1999, 2006). In addition to sucrose, analysis by high performance liquid chromatography (HPLC) detected glucose, fructose, and mannitol in snapdragon petals (Ichimura and Hisamatsu, 1999) as was reported in snapdragon leaves by Moore et al. (1997). Identification of mannitol was confirmed by Nuclear Magnetic Resonance analysis (Ichimura et al., 2005). Although treatment with mannitol somewhat extends the vase life of cut snapdragons, this treatment induces marked stem growth (Ichimura et al., 2005).

In the present study, we investigated the effects of various carbohydrates including mannitol on the vase life of cut snapdragon flowers. To clarify how mannitol promotes bud development and stem growth, we investigated the accumulation and metabolism of soluble carbohydrates as well as water and osmotic potential of spike tips.

2. Materials and methods

2.1. Plant material

Snapdragon (*Antirrhinum majus* L.) cv. Yellow Butterfly was grown under natural day-length conditions in a greenhouse (15 °C minimum and 25 °C maximum temperature). Flower spikes in which the most developed flower bud (3 cm in length) was expected to open the next day, were harvested in the morning and placed in tap water. They were immediately transported to a laboratory and used for experiments within 1 h.

2.2. Treatments with soluble carbohydrates

The flower spikes were recut to 30 cm and individually placed in a 300-mL glass vessel containing 250 mL of carbohydrate solution. In the other experiments, three flower spikes were placed in a 500-mL glass vessel containing 500 mL of carbohydrate solution. Mannitol concentration was set at 1, 10, 100, 250, or 500 mM, whereas glucose, sucrose, or sorbitol concentration was set at 250 mM because in a preliminary experiment, the most pronounced effect in mannitol treatment was observed at this concentration. All solutions including the control were supplemented with 200 mg L^{-1} 8-hydroxyquinoline sulfate (8-HQS) to inhibit microbial proliferation. The spikes were kept at 23 °C, 70% relative humidity, and 12-h light at 10 μmol m^{-2} s^{-1} from cool-white fluorescence lamps.

2.3. Measurements of flower bud number and spike length

The number of visible buds longer than 5 mm and open, wilted, and abscised flowers was scored daily. Flowers with fully unfolded petals were counted as open flowers. Flowers were considered to have terminated their life when the petals had wilted or abscised. The length of the flower spike was measured every 5 days. When stem length, bud number, and open flower number did not further increase, the measurements were completed. The total number of flower buds was scored 30 days after the start of treatment when the stem growth had completely ceased. Under a stereoscopic microscope, flower spike tips were dissected, and the buds with bracts were scored and included in the total number of flower buds.

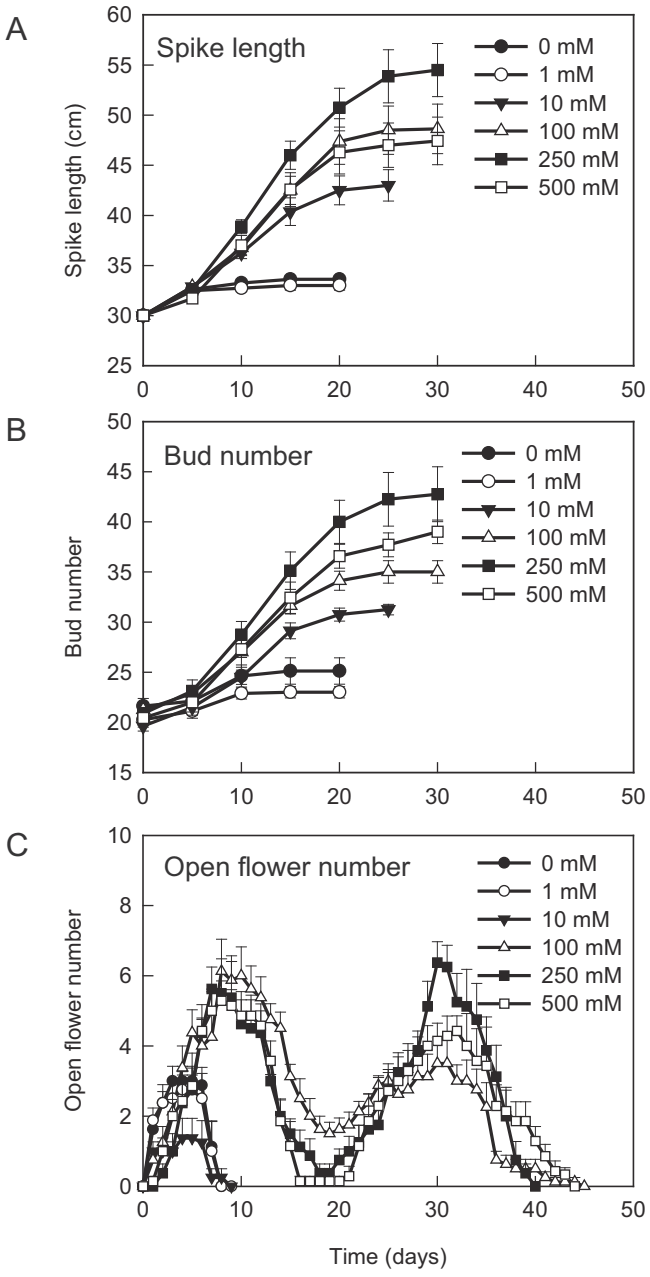

Fig. 1. Spike length (A), bud number (B), and open flower number (C) of cut snapdragons treated with mannitol at different concentrations. All solutions including the control contained 200 mg L^{-1} 8-HQS and cut flowers were held at 23 °C. The number of flower buds was the sum of the flower buds longer than 5 mm, open flowers, and wilted flowers. Values are the mean of 8 replicates ± SE (A and B) or +SE (C).